tory mechanisms may play roles in the aetiology of hip OA via an effect on cartilage.

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COMPARISON OF THE EXPRESSION PROFILES OF SYNOVIAL TISSUE INFLAMMATION BETWEEN END-STAGE AND PROGRESSIVE KNEE OSTEOARTHRITIS

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Purpose: An inflammation of the medial perimeniscal synovium is common in OA, as synovial inflammation in OA occurs locally in areas of cartilage loss. The synovial inflammation in early stage of knee OA is enhanced in comparison to that of late stage of OA. These suggest that the expression profiles of inflammatory proteins in synovium are different depending upon the condition of the knee joint such as the amount of a remaining articular cartilage and osteophyte formation. This study hypothesized that the expression patterns of the inflammatory mediators in the medial compartment of the patients with end-stage of knee OA (KL 4) that lack articular cartilage and show an eburnation of the medial contact surface is different from those with progressive stage (KL 2 and 3) of knee OA in which the articular cartilage remains, while the varus deformation and other clinical features of the former is exaggerated in comparison to that of the later. Therefore, the expression levels of inflammation mediators in the synovium of patients with the both end-stage and progressive stage of knee OA were examined to confirm the hypothesis.
Methods: Twenty-three patients who had knee OA (KL2 or higher) and underwent joint replacement (n=19) or arthroscopic surgery (n=4) in the hospital were recruited for this study. Synovial tissue samples of the 10 patients who underwent ACL reconstruction were also obtained as a control. The following primary antibodies were used in immunohistochemical analysis: CD31, NF-κB, MMP-1, COX-2, IL-1β, TGF-β. A semi-quantitative analysis of the stained sections was conducted using the method as described previously. Clinical manifestation was evaluated by the Japanese Knee Osteoarthritis Measure (JKOM). JKOM is higher in patients with more pain and physical disabilities.
Results: Immunohistochemical analyses have revealed the MMP-1, COX-2 and IL-1β expression in the synovium of end-stage OA were significantly reduced in comparison to those of progressive OA. On the other hand, the expression of TGF-β in the synovium of end-stage OA was significantly increased in comparison to that of progressive OA. The MMP-1, IL-1β and TGF-β expression in the synovium were significantly correlated with the radiographic and clinical manifestations of knee OA.
Conclusions: The expression of MMP-1, COX2 and IL-1β in synovial tissues is thought to be activated by degenerated articular cartilage. Therefore, the expression of these mediators of inflammation was enhanced in synovial tissues in the progressive OA in comparison to that in end-stage OA. The relationship between the expression levels of the mediators for inflammation and the radiographic and outcome measures for OA confirmed the accuracy of the immunohistochemical analysis. TGF-β is thought to be involved in osteophyte formation and fibrosis of the joint capsule in OA. The formation of an osteophyte is considered to be one of the signs of severe OA. Therefore, the result of TGF-β expression of the synovium in the patients with end-stage of knee OA in comparison to that with the progressive OA may reflect the severity of OA in this group in comparison to that in the progressive OA. These data indicated that the expression profiles of syniovial tissue inflammation in end-stage OA were distinct from those in progressive OA, reflecting the condition of the joint in patients with OA, such as the volume of the remaining articular cartilage and the degree of osteophyte formation.

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IL-1 SIGNALS BEYOND THE CANONICAL PATHWAY IN MURINE OA

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Purpose: Evaluation of knock-out (KO) mice in osteoarthritis (OA) models provides an excellent tool to validate OA targets *in vivo*. Interleukin-1β (IL-1β) is recognized as a critical cytokine, and is frequently utilized to induce a number of destructive enzymes and signaling molecules, implicated in the pathophysiology of OA. This study sought to elucidate the role of IL-1β, and its downstream signaling pathway, by utilizing KO mice in a surgical instability *in vivo* model, as well as *in vitro* explant and cell line studies.
Methods: All animal studies were performed with IACUC approvals. All KO mice were purchased commercially, bred from homozygous parents and the recommended wild-type (WT) controls were utilized. The IL-1β KO mice were purchased from Taconic (Germantown, NY), and the Interleukin-1 Receptor I (IL1RI), and Interleukin-1 Receptor Accessory Protein (IL1RaP) KO and control WT mice were from Jackson Laboraties (Bar Harbor, ME). Male mice (10 per group) underwent surgical destabilization of the medial meniscus (DMM) when 10 weeks of age, and were group housed until sacrificed 8-weeks later. Following euthanasia, right knees were collected for histopathology. Hip cartilage was harvested from 3-week-old IL1R1 and IL1RaP KO and WT mice for explant studies as well as for generation of cell lines. Explants were treated with media alone or IL-1. Cell lines were stimulated with media +/- 10 ng/ml IL-1β for 24 hours and RNA isolated for qRT-PCR of MMP-13, SOX9, ADAMTS4 and NOS2.
Results: The IL-1β KO had significantly less OA than the WT in 2 separate studies. No differences to WT were observed for IL1R1 (performed twice), or IL1RaP KOs. Both IL1R1 and IL1RaP KO explants were protected from IL-1 induced proteoglycan release. The IL1R1 and IL1RaP WT cell lines had increases in MMP-13, ADAMTS4 and NOS2, and decreases in SOX9, following IL-1 treatment. The IL1R1 and IL1RaP KO cell lines both had a lack of induction of MMP-13 following IL IL-1 treatment, yet SOX-9 repression and ADAMTS-4 induction remained similar to WT. The IL1R1 KO cell line had no induction of NOS2, following IL-1 stimulation, while the IL1RaP KO continued to have dramatic up-regulation.
Conclusions: The IL-1β KO was protected in the DMM model, supporting the importance of this cytokine in OA. However, deletion of downstream signaling molecules, IL1R1 or IL1RaP had no effect in the DMM model. Explant studies confirmed that IL1R1 or IL1RaP KOs did not respond to IL-1 treatment with release of proteoglycan. The cell lines studies found MMP-13 was suppressed, but unexpectedly ADAMTS-4 was still induced and SOX9 repressed in both KO cell lines, suggesting that IL-1β can act beyond the canonical signaling pathway in cartilage, in accordance with published studies in brain. These findings indicate that the role of IL-1β signaling in cartilage is more complicated than previously recognized and that studies utilizing KO mice and cell lines may be of great utility to explore the alternate mechanisms.